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**Development, spatial distribution and presence on grapevine of nymphs of  
*Orientus ishidae* (Matsumura), a new vector of Flavescence dorée phytoplasmas**

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**Abstract**

*Orientus ishidae* (Matsumura) (Cicadellidae: Deltocephalinae) is an Asian species now widespread in Europe, and a vector of 16SrV phytoplasmas agents of grapevine Flavescence dorée (FDP). Embryonic and post-embryonic development, spatial distribution, and relationships with grapevine of nymphs were studied under field and laboratory conditions. Egg hatching dynamics and post-embryonic development of nymphs were studied by collecting grapevine wood from managed and unmanaged vineyards (including both European *Vitis vinifera* L., and wild American rootstocks) and storing it inside rearing cages at T=21-23°C. Field sampling of nymphs were made on both grapevine and two elective host plants of *O. ishidae*: hazelnut and hornbeam. Taylor's Power Law was applied to assess the aggregation coefficient of early (first and second) and late (third to fifth) life instars on leaves and shoots of host plants. More nymphs were obtained from wood collected in unmanaged rather than managed vineyards. Under lab conditions, the embryonic development lasted 34 - 48 days, whereas the whole post-embryonic development averaged 27 days. Under field conditions, early instars peaked at the end of May, and late instars peaked 2-4 weeks later. The aggregation patterns decreased from early to late instars, and from leaves to shoots. Very few nymphs were observed on unmanaged grapevine, either European or American, and none on managed European grapevine. Some behavioral and FDP epidemiological consequences of the results obtained are discussed.

**Keywords:** grapevine, leafhopper, hatching, molt, Taylor's Power Law

27

## 28 **Introduction**

29 The Mosaic leafhopper *Orientus ishidae* (Matsumura) (Hemiptera: Cicadellidae: Deltocephalinae) is  
30 a new acknowledged vector of Flavescence dorée (FDP), a severe disease of grapevine caused by  
31 phytoplasmas in the 16SrV ribosomal group (Alma et al. 2015). The first clue occurred in 2010, when  
32 some adults have been found bearing 16SrV phytoplasmas in Slovenia (Mehle et al. 2010). The same  
33 results were then obtained in Northern Italy (Gaffuri et al. 2011). Meanwhile, the interest about this  
34 species increased progressively, and its presence throughout Europe was confirmed (Koczor et al.  
35 2013, Chireceanu et al. 2017, Klejdysz et al. 2017).

36 Its vector ability was proved by successful inoculation of 16SrV phytoplasmas to grapevines by  
37 adults, after an acquisition at the nymphal stage from infected broad beans (*Vicia faba* L.) and a  
38 latency period on hazelnut, under laboratory conditions (Lessio et al. 2016). Until then, FDP were  
39 thought being transmitted only by *Scaphoideus titanus* Ball (Cicadellidae: Deltocephalinae), a  
40 specialist of grapevine which is still acknowledged as the main vector (Chuche and Thiery 2014,  
41 Alma et al. 2015), and the European lantern fly *Dictyophara europaea* (L.) (Hemiptera:  
42 Dictyopharidae), an occasional vector (Lessio and Alma 2008, Filippin et al. 2009, Alma et al. 2015).  
43 *O. ishidae* has a single generation per year and overwinters in the egg stage (Nickel, 2010; Lessio et  
44 al., 2016). Adults are aggregated at the edges of vineyards, depending on host plants such as hazelnut,  
45 hornbeam, willow, and others (Lessio et al. 2016; Alma et al., 2019). Previously, some seasonal  
46 dynamics of adults and nymphs, along with the description of fifth-instars, were given in a survey on  
47 ornamental honey locust, *Gleditsia triacanthos* L. (Valley and Wheeler Jr 1985). However, many  
48 aspects of its biology are still unknown, especially concerning the embryonic and post-embryonic  
49 development of nymphs. Moreover, its relationships with grapevine are unclear, especially  
50 concerning egg-laying and population density of nymphs.

51 Given the lack of basic knowledge about this emerging pest, this research deals with several aspects  
52 of the biology of *O. ishidae* nymphs: presence of eggs in wood of grapevine, depending also on the

53 management of vineyards; embryonic and post-embryonic dynamics under laboratory conditions;  
54 seasonal occurrence of nymphs; presence of nymphs on wild or cultivated grapevine, compared to  
55 other elective host plants; spatial distribution of nymphs on elective host plants, at leaf and shoot  
56 levels.

57

58 **Materials and methods**

59 Study area

60 Samplings took place in 2017 and 2018, in vine growing areas settled within the following districts  
61 of Piedmont, North-western Italy: Caluso (45.31796 °N; 7.88061 °E), Mazzé (45.309539 °N;  
62 7.934383 °E), Borgiallo (45.399622 °N; 7.667674 °E), Portacomaro (44.962311 °N; 8.258457 °E),  
63 Mombercelli (44.820415 °N; 8.302749 °E), and Vesime (44.644452 °N; 8.226433 °E). Within these  
64 areas, we chose eight experimental sites: four managed (organic) vineyards; two abandoned vineyards  
65 (that is, let unpruned and unmanaged for a period of 2-5 years, with many weeds but without  
66 overgrown trees or vine rootstocks) (Camerano and Terzuolo, 2015); and two woods mainly  
67 consisting in wild hazelnut (*Corylus avellana* L.) and hornbeam (*Carpinus betulus* L.) trees, with  
68 overgrowing American rootstocks of *Vitis berlandieri* x *riparia* (e.g. Kober 5bb, SO4) and *Vitis*  
69 *berlandieri* x *rupestris* (e.g. 1003 Paulsen) (Camerano and Terzuolo, 2015), which are also a source  
70 of 16SrV phytoplasmas although often symptomless (Lessio et al. 2007). All of the sites were small-  
71 sized (1500-4000 m<sup>2</sup>). The vineyards were settled within an heterogeneous landscape consisting of  
72 many patches of broadleaf woods and edges of spontaneous hazelnut and hornbeam trees, which are  
73 two elective host plants of *O. ishidae* (Lessio et al. 2016). In organic vineyards, two insecticidal  
74 sprays were made by farmers with natural pyrethrum at the middle and end of June in both years,  
75 according to Regional Phytosanitary Service guidelines.

76

77 Egg-laying and hatching dynamics

78 To quantify egg-laying by *O. ishidae* on grapevine (*Vitis* spp.), and to study egg-hatching dynamics,  
79 wood was randomly collected in winter time from the each of the experimental sites (Table 1). Two-  
80 year (or more) old grapevine canes and branches were cut into pieces (15-20 cm), stored into a cool  
81 chamber (+5°C), and periodically sprinkled with water to avoid dehydration of eggs.  
82 At the end of January, the wood was placed indoors at an average temperature of  $T=22\pm1^{\circ}\text{C}$ , to start  
83 egg hatching. Each field-collected sample was weighted and placed inside a separate insect-proof  
84 cage (cm 110 x 110 x 80) made of mesh and aluminium frame, along with potted hazelnut and broad  
85 bean plants to provide food for nymphs. A layer of vermiculite was placed on the bottom of the cage  
86 to preserve humidity, and the cages were periodically sprinkled with water. The cages were inspected  
87 daily, and the emerged nymphs of *O. ishidae* were counted, removed and kept alive for the post-  
88 embryonic development experiments.

89

#### 90 Post-embryonic development

91 The post-embryonic development of *O. ishidae* was studied under the same conditions of the previous  
92 experiment. Newly hatched nymphs were retrieved from the rearing cage, and placed singularly inside  
93 Plexiglas cylinders (h=20 cm; diameter: 12 cm) closed with a fine mesh on the top. Two circular  
94 holes (diameter: 2 cm) were made on the walls of each cylinder, and covered with the same mesh to  
95 allow a better circulation of air. Each cylinder was placed onto a potted broad bean plant, and a disk  
96 of filter paper was placed on the soil to decrease humidity and for a better detection of any dead  
97 insect. Cylinders were inspected daily to observe moults up to the adult stage, and after each moult  
98 the corresponding *exuvia* was removed from inside.

99

#### 100 Sampling of nymphs on host plants

101 Nymphs of *O. ishidae* were sampled on European grapevine, on wild American rootstocks, and on  
102 surrounding spontaneous hazelnut and hornbeam plants, in all of the experimental sites. On the whole,  
103 N=79 plants were observed (European grapevine: 20; American rootstocks: 18; hazelnut: 33;

104 hornbeam: 8). Samplings took place from the middle of May to the end of July (2017-2018). On  
105 hazelnut and hornbeam, for each plant, we observed 50 leaves distributed on 10 shoots (5 leaves per  
106 shoot). On grapevine species, we inspected 50 leaves close to the trunk or to wooden canes. Leaves  
107 were gently turned upside down, and nymphs were counted under a lens without being removed,  
108 assigning them to I-II instar (N1 and N2: winglets absent), and III-V instar (N3, N4 and N5: winglets  
109 present).

110

### 111 Data analysis

112 Nymphs emerging from wood collected in different types of vineyards (cultivated or wild) in different  
113 years were compared with a GLM procedure. The number of nymphs was used as the dependent  
114 variable; the type of vineyard (either managed or unmanaged), the species of *Vitis* (either *V. vinifera*  
115 or American rootstocks) and the year of collection were the factors; and the weight of collected wood  
116 was the offset variable. To overcome problems due to zero values, we used a Tweedie mixed  
117 distribution, which manages properly both over-dispersion and zero-inflation, with a *Log* link  
118 function. A cumulative distribution function was used instead to describe egg hatching dynamics.  
119 Finally, we calculated the descriptive statistics (mean, standard deviation, and confidence limits) of  
120 the duration for both the embryonic development of nymphs emerged from different wood lots and  
121 post-embryonic development of each life instar.

122 The seasonal distributions of early (N1-2) and late (N3-5) instar of field-collected nymphs between  
123 the two sampling years were compared with a chi-square test with four degrees of freedom (five  
124 sampling dates, from the middle of May to the middle of July, at a bi-weekly step).

125 Counts of nymphs on plants were analyzed with a GLM procedure testing the effect of plant species  
126 (European grapevine, American rootstocks, hazelnut, hornbeam). In this case, we did not separate  
127 early from late instars nor we considered sampling date. The number of sample units per date and  
128 plant species was considered as an offset variable. A Poisson distribution of data was assumed, and  
129 the *Log* link function was used.

130 Taylor's Power Law (TPL) (Taylor 1984) was used to analyze data of nymphs distribution on leaves  
131 and shoots of hazelnut and hornbeam. The general equation of TPL is:

132 
$$\log_{10} S^2 = \log_{10} a + b \cdot \log_{10} m$$

133 where, having counted a given object (e.g. nymphs) on a given number of sampling units,  $S^2$  and  $m$   
134 are the sample variance and mean, respectively. While the coefficient  $a$  (intercept) depends just on  
135 the sampling method, the coefficient  $b$  (slope) is typical for the species considered:  $b < 1$  indicates  
136 randomness,  $b = 1$  uniformity, and  $b > 1$  aggregation. In this case, we considered first the leaves and  
137 then the shoots as a sampling unit, calculating therefore the mean and variance for each tree. In this  
138 case, we excluded counts on grapevines (no or too few nymphs detected, see results): therefore, a  
139 dataset of  $N=41$  was available. On the other hand, given the similar plant architecture (small broadleaf  
140 trees or bushes, with a relatively similar leaf size and distribution of leaves on the branches), we did  
141 not distinguish between counts on hornbeam or hazelnut. Data were analyzed separately depending  
142 on life instar (N1 and N2; N3, N4 and N5), and were previously Log10 transformed according to  
143 Taylor (1984) after adding a 0.1 constant to avoid transformation problems in zero values. Finally, a  
144 linear regression was run between the sample variance (dependent variable) and the sample mean  
145 (independent variable).

146 All of the statistical analyses listed were performed with SPSS 25.0® statistical package.

147

## 148 **Results**

### 149 Egg hatching and post-embryonic development

150 Collectively, 142 nymphs of *O. ishidae* were obtained from wood of grapevine, ranging 0.33 - 14.8  
151 per kg of wood in single collections. Overall, significant differences were found in the number of  
152 nymphs hatched from different kinds of wood collected in differently managed sites and during  
153 different years (GLM:  $\chi^2 = 40.01$ ;  $df=3$ ;  $P < 0.001$ ). When considering single factors, the type of  
154 vineyard had a significant influence on the number of nymphs hatched ( $\chi^2 = 16.86$ ;  $df=2$ ;  $P < 0.001$ ):  
155 organic vineyards were significantly lower than the other two kinds, whereas no differences were



156 detected between abandoned vineyards and woods with American rootstocks (Figure 1). No  
 157 differences were found between the two years of wood collection ( $\chi^2 = 0.81$ ;  $df=1$ ;  $P=0.37$ ).  
 158 The wood collected in 2017 in the sites of Caluso (abandoned vineyard; nymphs/kg wood: 14.78;  
 159 total: 17), Mazzé (wild American grapevine; nymphs/kg wood: 7.53; total: 29), Borgiallo (abandoned  
 160 vineyard; nymphs/kg wood: 8.83; total: 72), and Portacomaro (wild American grapevine; nymphs/kg  
 161 wood: 3.38; total: 13) provided enough nymphs to describe egg-hatching dynamics. Hatching started  
 162 from 2 to 4 weeks (13-30 d) after the beginning of incubation, and lasted 2-7 weeks (14-51 d). The  
 163 mean hatching time was minimum in Portacomaro (34 d) and maximum in Mazzé (48 d) (Table 2;  
 164 Figure 2).  
 165 Data of post-embryonic development under laboratory conditions were obtained from a set of  $N=20$   
 166 newly hatched nymphs, which were successfully reared up to the adult stage (other specimens died  
 167 before becoming adults and were therefore excluded from analysis). Developmental times increased  
 168 along with life stages: N1 lasted approx. 4-5 days, whereas N5 lasted 6-7 days. Overall, the post-  
 169 embryonic development lasted 27-29 days (Table 3).

#### 171 Seasonal dynamics of nymphs on host plants

172 Collectively, 507 nymphs were observed on hazelnut and hornbeam plants throughout different  
 173 sampling periods (N1-N2: 285; N3-N5: 222). The first nymphs (N1-2) appeared at the middle of May,  
 174 whereas N3-5 appeared between the end of May and the middle of June. The peak of N1-2 occurred  
 175 at the end of May in both years, whereas N3-5 peaked at the end of June in 2017 and at the middle of  
 176 June in 2018. At the end of July, no nymphs were found. Data are shown in Figure 3. Referring to the  
 177 distribution among five sampling dates (from the middle of May to the middle of July), no differences  
 178 were found between years, neither for early stages ( $\chi^2 = 0.22$ ;  $df=4$ ;  $P=0.05$ ), nor for late ones ( $\chi^2$   
 179  $=0.42$ ;  $df=4$ ;  $P=0.09$ ). On the other hand, only three specimens were observed on grapevine  
 180 (abandoned grapevines: 1 early instar on May 31, 2017, and 1 late instar on June 14, 2017; wild  
 181 grapevine: 1 late instar on June 16, 2018). A significant difference was observed in the number of

182 nymphs detected on different plant species (GLM:  $\chi^2= 3971.05$ ;  $df=3$ ;  $P<0.001$ ). The number of  
183 nymphs per plant was significantly higher on hazelnut with respect to all other. As well, it was  
184 significantly higher on hornbeam with respect to both species of grapevine, whereas no significant  
185 differences were found between European and American grapevine (Table 4).

186

#### 187 Aggregation patterns of nymphs

188 The aggregation patterns of *O. ishidae* nymphs, calculated with TPL, resulted highly significant and  
189 changed depending on the instar and the plant organ considered as sample unit (Table 5). When  
190 considering leaves, early life instars were indicating a moderately aggregated ( $b=1.29$ ), whereas late  
191 ones resulted less crowded ( $b=1.16$ ). The same trend was observed for spatial distribution at a shoot  
192 level, however both early and late instars were less aggregated with respect to leaves. In particular,  
193 late instars on shoots had an almost uniform spatial distribution ( $b=1.07$ ).

194

#### 195 **Discussion**

196 The present research confirmed that *O. ishidae* is capable of laying eggs on grapevine. This behavior  
197 may be promoted by the proximity of trees to vineyards or by the co-habitat of wild grapevine and  
198 other plants in woods. In fact, more nymphs hatched from wood collected from abandoned vineyards  
199 and/or wild rootstocks, where hazelnut and hornbeam plants were generally closer to the edges or, in  
200 the case of woods, mingled with overgrowing grapevine rootstocks. Another reason may be the  
201 greater presence of older wood (2 years, or more) due to no pruning. Although we did not take into  
202 account 1-year old wood for the presence of eggs, it is likely that *O. ishidae* prefers older grapevine  
203 wood just like *S. titanus* (Lessio and Alma 2013, Chuche and Thiery 2014). Therefore, females might  
204 have fed and developed on other plants and only afterwards exploited grapevine wood for egg-laying.  
205 However, while this species was very abundant on hornbeam and hazelnut, very few nymphs were  
206 found on grapevine leaves. Although in organic vineyards pyrethrum was sprayed on grapevines after  
207 the middle of June, nymphs were not found even before treatments. Therefore, there must be another

208 reason to explain such a discrepancy. Perhaps, grapevine is used just for egg-laying, and nymphs  
209 move elsewhere after hatching. Among leafhoppers in the same subfamily (Deltocephalinae),  
210 *Anoplotettix fuscovenosus* (Ferrari) exhibits a similar strategy: eggs are laid under the bark of  
211 grapevine, but nymphs move to the weeds in the inter-row (Alma 1995). Sometimes, *Phlogotettix*  
212 *cyclops* (Mulsant & Rey) lays eggs under the bark of grapevine canes too (Chuche et al. 2010). It is  
213 not surprising therefore that females of *O. ishidae* exploit grapevines for egg-laying, given also their  
214 frequent drifting behaviour in vineyards (Lessio et al. 2016). However, it is not clear if egg-laying on  
215 grapevines represents a biological strategy or just a casual occurrence. In any case, although nymphs  
216 of *O. ishidae* are capable of acquiring 16SrV phytoplasmas from grapevines (Lessio et al. 2016), this  
217 aspect does not seem important from an epidemiological point of view, as very few specimens have  
218 been found on grapevines.

219 The embryonic development of *O. ishidae* lasted 34-48 days at T=21-23°C. A similar trend has been  
220 observed in *S. titanus*, which has a mean hatching time of 30 and 45 days at constant temperatures of  
221 20 and 22°C, respectively (Falzoi et al. 2014). The discrepancies observed in the egg-hatching  
222 dynamics among sites may be due to cold or mild winters. In fact, it has been demonstrated that *S.*  
223 *titanus* eggs hatch faster when exposed to cold rather than mild winter temperatures (Chuche and  
224 Thiery 2009). Concerning the post-embryonic development, late instars of *O. ishidae* take longer to  
225 develop than early ones: this is similar too to *S. titanus* (Falzoi et al. 2014), and is related to the fact  
226 that body size has an effect on developmental time (Gillooly et al. 2002).

227 Seasonal dynamics of nymphs observed in the field were partially in accordance with the data  
228 presented by Valley and Wheeler jr (1985), who found them from the beginning to the end of June  
229 on ornamental honey locust in Pennsylvania. In our research, nymphs were found from the middle of  
230 May up to the middle of July. These differences may be due to different temperatures between  
231 Pennsylvania and Northern Italy, to increasing temperatures over the last three decades, to differences  
232 in host plants, or simply to sampling discrepancies. With regard to the influence of host plants, it is  
233 possible that *O. ishidae* populations have adapted to differences in bud break among plant species.

234 This synchrony has been detected concerning grapevine and *S. titanus* (Chuche et al. 2015), which  
235 however is a monophagous species and is probably co-evolving with its own host plant. *O. ishidae* is  
236 highly polyphagous (Nickel, 2010; Lessio et al., 2016; Alma et al., 2019), and therefore it is less  
237 likely that populations specialize on one host plant: in fact, the success in this species is probably due  
238 to its plasticity.

239 The spatial distribution of nymphs was different considering both the life stage and the sampling unit.  
240 Dispersal increased from early to late instars, and from leaves to shoots. Late instars are less  
241 aggregated probably because they disperse due to overcrowding on leaves. This aspect is not observed  
242 in *S. titanus*, which rarely builds up great densities on grapevine leaves. In fact, the nymphs of this  
243 species are aggregated (Lessio and Alma 2006), and have also an aggregative feeding behaviour of  
244 under laboratory conditions (Chuche et al. 2009). This could be due to differences in plant  
245 architecture. In fact, grapevine (especially if row-shaped) has few shoots sprouting from the trunk:  
246 nymphs of *S. titanus* hatching from eggs laid under the bark have therefore less shoots to colonize.  
247 On the other hand, nymphs of *O. ishidae* have more possibilities of reaching sprouts when eggs are  
248 laid on the trunk and on the branches of broadleaf trees. Another reason may be the different feeding  
249 habits between these two species. *O. ishidae* causes severe stunting on leaves (Felt and Bromley 1941,  
250 Lessio et al. 2016), probably because of a cell rupture feeding behavior. In fact, damages resemble in  
251 some way marginal burning caused by *Empoasca vitis* (Goethe), a typical cell rupture feeder (Jin et  
252 al. 2012). Therefore, overcrowding may cause a decrease of food resources. On the other hand, *S.*  
253 *titanus* usually probes in one point producing salivary sheaths (Chuche et al. 2017), without affecting  
254 directly grapevine leaves.

255 The similarity of life cycles between *S. titanus* and *O. ishidae* may be the reason why both are vectors  
256 of 16SrV phytoplasmas to grapevine, although with different efficiency and therefore importance.  
257 However, phytoplasma sources for nymphs of *O. ishidae* are less certain. No nymphs collected on  
258 many host plants tested FD-positive, although they are capable of acquiring from infected grapevines  
259 (Lessio et al. 2016). Recently, some host plants (e.g. willow, hazelnut) have been found positive to

260 16SrV phytoplasmas in Switzerland (Casati et al. 2017). Another possibility is that adults of *O.*  
261 *ishidae* acquire phytoplasmas directly on grapevine. In fact, it has recently been proved that *S. titanus*  
262 is capable of acquiring 16SrV phytoplasmas in the adult stage, and transmitting them within only two  
263 weeks (Alma et al. 2018). Given the biological similarities between *S. titanus* and *O. ishidae*, this  
264 matter should be further investigated.

265  
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268  
269 **Author's contribution statement**

270 FL designed the experiments, made field samplings, conducted laboratory rearing and tests, analyzed  
271 data, and wrote the manuscript. FB made field samplings and analyzed data. AA conceived and  
272 designed the research. All authors read and approved the manuscript.

273  
274 **Compliance with Ethical Standards**

275 Conflict of interest: all Authors declare that they have no conflict of interest.  
276 Ethical approval: this article does not contain any studies with human participants performed by any  
277 of the Authors; all applicable international, national and institutional guidelines for the care and use  
278 of animals were followed; no unauthorized sampling of wildlife forms was performed;

279  
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**Table 1.** Wood of grapevine (*Vitis* spp.) collected in different experimental sites

Site	District	Management type	<i>Vitis</i> species	year	Kg wood collected
1	Mazzé	Wood	American rootstocks	2017	3.71
				2018	1.00
2	Caluso	Abandoned vineyard	<i>V. vinifera</i>	2017	1.50
				2018	1.00
3	Borgiallo	Abandoned vineyard	<i>V. vinifera</i>	2017	2.00
				2018	6.90
4	Portacomaro	Wood	American rootstocks	2017	4.00
				2018	1.15
5	Mongardino	Organic vineyard	<i>V. vinifera</i>	2017	2.00
				2018	6.00
6	Caluso	Organic vineyard	<i>V. vinifera</i>	2017	5.00
				2018	9.00
7	Mazzé	Organic vineyard	<i>V. vinifera</i>	2017	5.00
				2018	4.00
8	Vesime	Organic vineyard	<i>V. vinifera</i>	2017	5.00
				2018	5.00

**Table 2.** Time of embryonic development (TD, in days) in *O. ishidae* at T=21-23°C

Site	Year	N	TD <sub>min</sub>	TD <sub>max</sub>	HD	TD <sub>mean</sub>	SE	Lower CI	Upper CI
1	2017	30	25	59	34	48.87	0.34	48.20	49.54
2	2017	17	30	50	20	39.76	0.30	39.17	40.36
3	2017	72	13	64	51	46.36	0.15	46.08	46.65
4	2017	13	28	42	14	34.46	0.39	33.69	35.23

N: number of hatched nymphs; TD<sub>min</sub> and TD<sub>max</sub>: time of development of the first and last hatched specimen; HD: hatching duration ( $HD = TD_{max} - TD_{min}$ ); SE: standard error; CI: 95% confidence interval. Site 1: Mazzè (unmanaged, Am. grapevine); site 2: Caluso (unmanaged, Eur. grapevine); site 3: Borgiallo (unmanaged, Eur. grapevine); site 4: Portacomaro (unmanaged, Am. grapevine).

**Table 3.** Duration of post-embryonic development (in days) in *O. ishidae* (N=20) at T=21-23°C

Life stage	Mean	SE	Lower CI	Upper CI
N1	4.45	0.18	4.09	4.81
N2	5.30	0.46	4.40	6.20
N3	5.30	0.29	4.73	5.87
N4	5.85	0.17	5.52	6.18
N5	6.95	0.34	6.28	7.62
Total	27.85	0.55	26.76	28.94

SE: standard error; CI: 95% confidence interval.

**Table 4.** Nymphs of *O. ishidae* counted on different host plants. Different letters indicate significant differences (GLM,  $P < 0.05$ ).

Plant species	Nymphs (mean $\pm$ s.e.)	$\chi^2$ (d.f.)	<i>P</i>
<i>Corylus avellana</i> L.	13.00 $\pm$ 1.32 a	3971.05 (3)	<0.001
<i>Carpinus betulus</i> L.	9.38 $\pm$ 2.68 b		
<i>Vitis vinifera</i> L.	0.10 $\pm$ 0.07 c		
American rootstocks	0.06 $\pm$ 0.05 c		

**Table 5.** Taylor's Power Law regressions on nymphs of *O. ishidae* counted on leaves and shoots of broadleaf host plants

Source of variation	$R^2$	ANOVA			Coefficients		
		$F_{(1, 39)}$	$P$		B	t	$P$
N1+N2, leaves	0.96	894.75	0.000	Intercept	0.52	9.72	0.000
				Slope	1.29	29.91	0.000
N3+N4+N5, leaves	0.95	726.38	0.000	Intercept	0.29	5.41	0.000
				Slope	1.16	26.95	0.000
N1+N2, shoots	0.93	510.06	0.000	Intercept	0.25	5.03	0.000
				Slope	1.16	22.58	0.000
N3+N4+N5, shoots	0.93	528.31	0.000	Intercept	0.13	2.92	0.01
				Slope	1.07	22.99	0.000

### Captions to figures

**Figure 1.** Nymphs of *O. ishidae* (mean  $\pm$  s.e. per kg of wood) hatched from different kinds of grapevine's wood. Different letters indicate significant differences in wood type (GLM,  $P < 0.05$ )

**Figure 2.** Cumulative frequency distribution of egg-hatching in *O. ishidae* obtained from grapevine wood at  $T = 21-23^{\circ}\text{C}$

**Figure 3.** Seasonal distribution of nymphs in *O. ishidae* collected on host plants (hazelnut and hornbeam). A: 2017; B: 2018